植 物 分 类 与 资 源 学 报 2014, **36** (4): 545~550
Plant Diversity and Resources DOI: 10.7677/ynzwyj201413182

黄皮根和茎中一个新的木脂体*

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摘要: 从黄皮 (*Clausena lansium*) 根和茎中分离得到 8 个化合物, 其中化合物 claulignan (**1**) 为一个新的木脂体类化合物, 通过 1D-、2D-NMR 和高分辨质谱确定其结构。化合物 **2-8** 首次从黄皮属 (*Clausena*) 植物中分离得到。活性筛选结果表明, 化合物 **1** 和 **6** 显示一定的细胞毒活性, 对人宫颈癌 HeLa 细胞株的 IC_{50}分别为 25.03 和 53.99 μM; 化合物 **3** 具有一定的抗氧化活性, 在 DPPH 实验中的 EC_{50}为 268.96 g·kg^{-1}。
关键词: 黄皮; 木脂体; 细胞毒; DPPH; Claulignan
中图分类号: Q 946 **文献标识码**: A **文章编号**: 2095-0845(2014)04-545-06

A New Cytotoxic Oxyneolignan from the Roots and Stems of *Clausena lansium* (Rutaceae)*

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Abstract: A new oxyneolignan, claulignan (**1**), together with seven known compounds (**2-8**), were isolated from the methanol extract of the roots and stems of *Clausena lansium*. Their structures were elucidated on the basis of 1D- and 2D-NMR experiments and mass spectrometry. Compounds **2-8** were isolated from the genus *Clausena* for the first time. Compounds **1** and **6** showed cytotoxicity against human cervical cancer HeLa cell line with the IC_{50} values of 25.03 and 53.99 μM, respectively. Compound **3** exhibited antioxidant activity in the DPPH assay with the EC_{50} value of 268.96 g·kg^{-1}.
Key words: *Clausena lansium*; Oxyneolignan; Cytotoxicity; DPPH; Claulignan

Clausena belongs to the family Rutaceae and includes about 10 species in China. *Clausena lansium* (Lour.) Skeels is a fruit tree and widely distributes in southern China (Huang, 1997). Its fruits have been used for treating indigestion, cold, cough, and stomach pain; its seeds for treating acute, chronic gastrointestinal inflammation, and ulcer; and its leaves and roots for the treatment of cough, asthma, dermatological disease, viral hepatitis, and gastro-intestinal disease (Shen *et al.*, 2012). Previous phytochemical investigation revealed that *C. lansium* contained flavonoid glycosides, coumarins, cabazole alkaloids, amides, and terpenoids with cytotoxic and neuroprotective activities (Ito *et al*, 1998; Lakshmi *et al.*, 1989; Liu *et al.*, 2012; Maneerat *et al.*, 2012; Yang *et al.*, 1988; Zhao *et*

* Funding: Found of the National New Drug Innovation Major Project of China (2011ZX09307-002-02), the National Natural Science Foundation of China (U1032602 and 91013002), the National Basic Research Program of China (2009CB522300), the Fund of Chinese Academy of Sciences (Hundred Talents Program), and the Natural Science Foundation of Yunnan Province (2012GA003)
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Received date: 2013-09-16, Accepted date: 2013-10-10
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al., 2010). In order to discover bioactive constituents, the methanol extract of the roots and stems of *C. lansium* was studied. Herein we report the isolation and structural elucidation of one new oxyneolignan and 7 known compounds from this extract with cytotoxic and antioxidant activities.

1 Results and discussion

Claulignan (**1**) was obtained as a brown powder and its molecular formula was deduced as $C_{20}H_{22}O_7$ on the basis of HR-EI-MS at *m/z* 374.1378 $[M]^+$ (calcd for $C_{20}H_{22}O_7$, 374.1366). The ^{13}C-NMR spectrum of **1** (Table 1) displayed ten carbon signals, including six aromatic carbons (δ_C 152.3, 148.3, 130.6, 124.0, 115.4 and 111.5), two methylene carbons (δ_C 58.7 and 41.5), one keto-carbon (δ_C 198.2) and one methoxy carbon (δ_C 56.2). In the ^{1}H-NMR spectrum of **1** (Table 1), three aromatic proton signals at δ_H 7.55 (d, J=1.5 Hz), 6.92 (d, J=8.2 Hz) and 7.59 (dd, J=8.2, 1.5 Hz) were observed. These data indicated that **1** might be an oxyneolignan with the same unit of 3, 4-disubstituted phenylpropanoid. Position of the keto-group was determined by the HMBC correlations from H-2, H-6, H-8 and H-9 to C-7, and the COSY correlation between H-8 and H-9 (Fig. 1). The HMBC correlation from $OC\underline{H}_3$ to C-3 and the ROESY correlation between H-2 and $OC\underline{H}_3$ suggested that the methoxy group linked at C-3 and the two same phenylpropanoid units linked at C-4 (Fig. 2). Thus, the structure of **1** was established.

Seven known compounds were identified to be clemaphenol A (**2**) (Song *et al.*, 2010), syringaresinol (**3**) (Ouyang *et al.*, 2007), ficusol (**4**) (Li and Kuo, 1998), berfussinol (**5**) (Kim *et al.*, 2011), 4-hydroxy-2-methoxycinnamaldehyde (**6**) (Xu and Wang, 2011), 3-methoxy-4-hydroxybenzaldehyde (**7**) (Yang *et al.*, 2007) and 4-hydroxybenzaldehyde (**8**) (Feng *et al.*, 2008) (Fig. 3). Compounds **2**–**8** were isolated from the genus *Clausena* for the first time. Compounds **1** and **6** showed cytotoxicity against human cervical cancer HeLa cell line with the IC_{50} values of 25.03 and 53.99 μM, respectively. Compound **3** exhibited antioxidant activity in the DPPH assay with the EC_{50} value of 268.96 $g \cdot kg^{-1}$.

Table 1 ^{1}H and ^{13}C NMR data for compound **1** in acetone-d_6 (δ in ppm, J in Hz)

NO.	δ_C [a)]	δ_H [b)]
1, 1′	130.6 (s)	
2, 2′	111.5 (d)	7.55 (d, 1.5)
3, 3′	148.3 (s)	
4, 4′	152.3 (s)	
5, 5′	115.4 (d)	6.92 (d, 8.2)
6, 6′	124.0 (d)	7.59 (dd, 8.2, 1.5)
7, 7′	198.2 (s)	
8, 8′	41.5 (t)	3.16 (m)
9, 9′	58.7 (t)	3.91 (overlapped)
OH		8.70 (s)
OMe	56.2 (q)	3.90 (s)

a) 600 MHz; b) 100 MHz

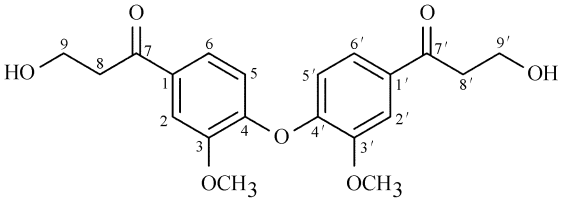


Fig. 1 Structure of compound **1**

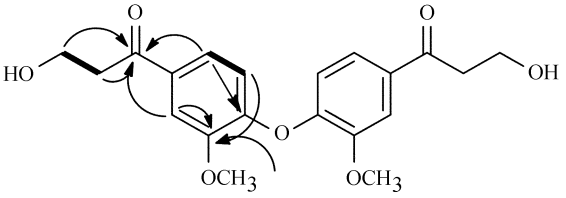
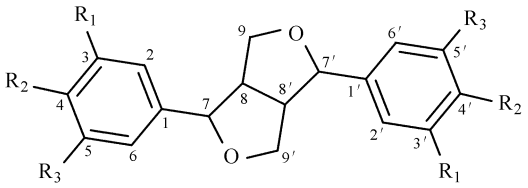


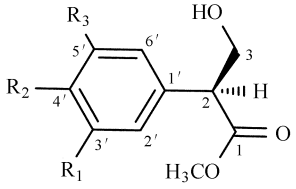
Fig. 2 The key HMBC (⟶) and ^{1}H, ^{1}H-COSY (—) correlations for compound **1**

2 Experimental

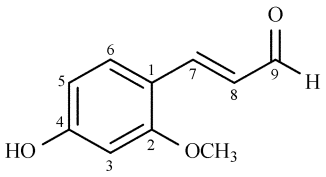
General experimental procedures Optical rotation was measured on a Jasco P-1020 polarimeter. UV spectrum was obtained from shimadzu UV-2401PC spectrophotometer. IR spectrum was recorded on a BRUKER Tensor 27 FT-IR spectrometer with KBr pellets. The 1D- and 2D-NMR spectra were



2 R_1=OH, R_2=OCH_3, R_3=H
3 R_1=R_3=OCH_3, R_2=OH



4 R_1=OCH_3, R_2=OH, R_3=H
5 R_1=R_3=OCH_3, R_2=OH



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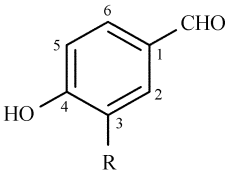

7 R=OCH_3
8 R=H

Fig. 3　Structures of compounds **2–8**

obtained from Bruker AM-400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) or Bruker DRX-500 (^{1}H: 500 MHz, ^{13}C: 150 MHz) or Bruker AV-III 600 (^{1}H: 600 MHz, ^{13}C: 150 MHz) spectrometer in acetone-d_6, CD_3OD or pyridine-d_5 at the room temperature. ESI-MS was determined on a Waters Xevo TQ-S or Bruker HCT/Esquire. EI-MS and HR-EI-MS were determined on a Waters AutoSpec Premier P776. Silica gel (200–300 mesh; Qingdao Yuminyuan Silica Reagent Factory, Qingdao, P. R. China), RP-18 (40–60 μm, Merck, Darmstadt, Germany), and MCI gel (CHP-20P, 70–150 μm, Mitsubishi Chemical Corporation, Japan) were used for column chromatography (CC). Fractions were monitored by TLC (Silica gel GF_{254} Plates, Qingdao Yuminyuan Silica Reagent Factory, Qingdao, P. R. China) and spots were visualized by UV light (254 and 365 nm), and by spraying with 5% aq. H_2SO_4 soln. followed by heating. MPLC, equipped with a UV detector (EZ Purifier 100/200, Lisure Science (Suzhou) Co., LTD. P. R. China) and a RP-18 column (3.5×25 or 4.5×40 cm) or a MCI column (4.5×40 cm) at a flow rate of 25 mL·min^{-1}, and preparative reversed-phase HPLC, Agilent 1100 apparatus equipped with a UV detector and a SunFire OBD (Waters, 1.9×25 cm, 5 μm) column at a flow rate of 10 mL·min^{-1} were used for purification.

Plant material　The roots and stems of *Clausena lansium* were collected from Hekou, Honghe Hani & Yi Autonomous Prefecture, Yunnan province, P. R. China, in September 2010 and identified by Prof. Yu-Min Shui, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 0599043) was deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation　Air dried, powdered roots and stems of *C. lansium* (27 kg) were extracted and refluxed with MeOH for 3 times each 4 hours (MeOH, 50 L×3). The extract was evaporated under reduced pressure to yield a dark brown residue (900 g). The residue was suspended in MeOH/H_2O (7∶3, 3 000 mL) and then partitioned with EtOAc (3×2 000 mL). After removing solvent, the EtOAc-soluble part (406 g) was fractionated by silica gel (200–300 mesh) column chromatograph (CC) and eluted with $CHCl_3$-MeOH (30∶1–4∶1) to afford 6 fractions, Fractions 1–6 (Fr. 1–6), on the basis of

TLC analysis. Fr. 3 (25 g) was chromatographed over MPLC with MCI ($MeOH/H_2O$ 10∶90→100∶0) and silica gel (petroleum ether/acetone 15∶1→7∶3) successively to afford **6** (15 mg) and **7** (10 mg). Fr. 4 (17.9 g) was firstly subjected to MPLC with MCI column ($MeOH/H_2O$ 10∶90→60∶40) to remove pigment and then to silica gel CC (petroleum ether/acetone 9∶1) and preparative HPLC (acetonitrile/H_2O 55∶45) to afford **2** (26 mg). MPLC chromatograph of Fr. 5 (6.9 g) over MCI ($MeOH/H_2O$ 5∶95→60∶40) afforded 7 sub-fractions: Fr. 5-1-Fr. 5-7. Fr. 5-1 was repeatedly chromatographed over silica gel (petroleum ether/acetone 9∶1) and then subjected to preparative HPLC (acetonitrile/H_2O 55∶45) to afford **1** (22 mg), **3** (280 mg), **4** (94 mg) and **5** (35 mg). Fr. 6 (77 g) was subjected to silica gel CC ($CHCl_3$/acetone 15∶1→7∶3), silica gel CC (petroleum ether/acetone 5∶1), MPLC with MCI ($MeOH/H_2O$ 10∶90→60∶40), MPLC with RP-18 ($MeOH/H_2O$ 5∶95→70∶30) and silica gel CC (petroleum ether/EtOAc 5∶1), subsequently to afford **8** (21 mg).

Claulignan (**1**) was obtained as brown powder, $C_{20}H_{22}O_7$; $[\alpha]_D^{21.0} = -12.93$ ($c = 0.10$, MeOH); UV (MeOH) λ_{max} (log ε): 205 (4.48), 228 (4.48), 276 (4.30), 303 (4.23) nm; IR (KBr) ν_{max} (cm^{-1}): 3423, 2960, 1665, 1591, 1517, 1464, 1427, 1385, 1279, 1197, 1167, 1136, 1032; ^1H NMR (acetone-d_6, 600 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz): see Table 1; ESI-MS (positive) *m/z*: 397 $[M+Na]^+$, 771 $[2M+Na]^+$; HR-EI-MS *m/z*: 374.1378 $[M]^+$ (calcd. for $C_{20}H_{22}O_7^+$, 374.1366).

Clemaphenol A (**2**) was obtained as colorless oil, $C_{20}H_{22}O_6$; $[\alpha]_D^{23.7} = +0.16$ ($c = 0.42$, MeOH); ^1H NMR (400 MHz, acetone-d_6): δ_H 6.98 (2H, d, $J = 1.3$ Hz, H-2, H-2′), 6.83 (1H, dd, $J = 8.1$, 1.3 Hz, H-6, H-6′), 6.78 (2H, d, $J = 8.1$ Hz, H-5, H-5′), 4.65 (2H, d, $J = 3.8$ Hz, H-7, H-7′), 4.20 (2H, m, H_a-9, H_a-9′), 3.83 (6H, s, OCH_3), 3.80 (2H, dd, $J = 9.2$, 3.8 Hz, H_b-9, H_b-9′), 3.10 (2H, m, H-8, H-8′); ^{13}C NMR (100 MHz, acetone-d_6): δ_C 148.2 (s, C-4, C-4′), 146.8 (s, C-3, C-3′), 134.1 (s, C-1, C-1′), 119.6 (d, C-6, C-6′), 115.5 (d, C-5, C-5′), 110.5 (d, C-2, C-2′), 86.6 (d, C-7, C-7′), 72.1 (t, C-9, C-9′), 56.2 (q, OCH_3), 55.2 (d, C-8, C-8′); ESI-MS (positive) *m/z*: 381 $[M+Na]^+$, 739 $[2M+Na]^+$. (Song *et al.*, 2010)

Syringaresinol (**3**) was obtained as colorless oil, $C_{22}H_{26}O_8$; $[\alpha]_D^{21.5} = -6.7$ ($c = 0.20$, MeOH); ^1H NMR (400 MHz, CD_3OD): δ_H 6.51 (4H, s, H-2, H-2′, H-6, H-6′), 4.54 (2H, d, $J = 3.8$ Hz, H-7, H-7′), 4.09 (2H, m, H_a-9, H_a-9′), 3.71 (2H, dd, $J = 9.3$, 2.8 Hz, H_b-9, H_b-9′), 3.67 (12H, s, OCH_3), 2.97 (2H, br. s, H-8, H-8′); ^{13}C NMR (100 MHz, CD_3OD): δ_C 149.1 (s, C-3, C-3′, C-5, C-5′), 135.9 (s, C-4, C-4′), 133.0 (s, C-1, C-1′), 104.3 (d, C-2, C-2′, C-6, C-6′), 87.4 (d, C-7, C-7′), 72.6 (t, C-9, C-9′), 56.7 (q, OCH_3), 55.3 (d, C-8, C-8′); ESI-MS (negative) *m/z*: 417 $[M-H]^-$, 418 $[M]^-$. (Ouyang *et al.*, 2007)

Ficusol (**4**) was obtained as colorless oil, $C_{11}H_{14}O_5$; $[\alpha]_D^{21.7} = -5.5$ ($c = 0.38$, MeOH); ^1H NMR (400 MHz, acetone-d_6): δ_H 6.92 (1H, d, $J = 0.8$ Hz, H-2′), 6.77 (2H, overlapped, H-5′, H-6′), 4.07 (1H, m, H_a-3), 3.82 (3H, s, 3′-OCH_3), 3.74 (1H, overlapped, H_b-3), 3.68 (1H, overlapped, H-2), 3.63 (3H, s, 1-OCH_3); ^{13}C NMR (100 MHz, acetone-d_6): δ_C 174.1 (s, C-1), 148.3 (s, C-3′), 146.8 (s, C-4′), 128.6 (s, C-1′), 121.5 (d, C-6′), 115.8 (d, C-5′), 112.5 (d, C-2′), 65.2 (t, C-3), 56.2 (q, 3′-OCH_3), 54.9 (d, C-2), 52.0 (q, 1-OCH_3); ESI-MS (positive) *m/z*: 249 $[M+Na]^+$. (Li and Kuo, 1998)

Berfussinol (**5**) was obtained as white solid, $C_{12}H_{16}O_6$; $[\alpha]_D^{21.7} = -5.8$ ($c = 0.15$, MeOH); ^1H NMR (400 MHz, acetone-d_6): δ_H 6.60 (2H, s, H-2′, H-6′), 4.07 (1H, m, H_a-3), 3.79 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.71 (1H, overlapped,

H_b-3), 3.70 (1H, overlapped, H-2), 3.63 (3H, s, 1-OCH_3); ^{13}C NMR (100 MHz, acetone-d_6): δ_C 174.0 (s, C-1), 148.6 (s, C-3′, C-5′), 136.2 (s, C-4′), 127.5 (s, C-1′), 106.5 (d, C-2′, C-6′), 65.2 (t, C-3), 56.6 (q, 3′-OCH_3, 5′-OCH_3), 55.1 (d, C-2), 51.9 (q, 1-OCH_3). ESI-MS (positive) *m/z*: 279 $[M+Na]^+$, 535 $[2M+Na]^+$. (Kim *et al.*, 2011)

4-Hydroxy-2-methoxycinnamaldehyde (**6**) was obtained as yellow oil, $C_{10}H_{10}O_3$; ^{1}H NMR (600 MHz, acetone-d_6): δ_H 9.63 (1H, d, J=7.8 Hz, H-9), 8.43 (1H, s, OH), 7.57 (1H, d, J=15.8 Hz, H-7), 7.39 (1H, d, J=2.0 Hz, H-3), 7.21 (1H, dd, J=8.0, 2.0 Hz, H-5), 6.91 (1H, d, J=8.0 Hz, H-6), 6.67 (1H, dd, J=15.8, 7.8 Hz, H-8), 3.92 (3H, s, OCH_3); ^{13}C NMR (150 MHz, acetone-d_6): δ_C 194.0 (d, C-9), 154.2 (d, C-7), 150.9 (s, C-4), 148.9 (s, C-2), 127.4 (s, C-1), 127.0 (d, C-8), 124.9 (d, C-6), 116.2 (d, C-5), 111.4 (d, C-3), 56.3 (q, OCH_3); ESI-MS (negative) *m/z*: 177 $[M-H]^-$, 178 $[M]^-$, 355 $[2M-H]^-$. (Xu and Wang, 2011)

3-Methoxy-4-hydroxybenzaldehyde (**7**) was obtained as white powder, $C_8H_8O_3$; ^{1}H NMR (600 MHz, acetone-d_6): δ_H 9.83 (1H, s, CHO), 8.77 (1H, s, OH), 7.46 (1H, dd, J=7.9, 1.8 Hz, H-6), 7.44 (1H, d, J=1.8 Hz, H-2), 7.01 (1H, d, J=7.9 Hz, H-5), 3.93 (3H, s, OCH_3); ^{13}C NMR (150 MHz, acetone-d_6): δ_C 191.2 (d, CHO), 153.6 (s, C-4), 149.0 (s, C-3), 130.8 (s, C-1), 127.10 (d, C-6), 116.0 (d, C-5), 110.8 (d, C-2), 56.3 (q, OCH_3); ESI-MS (negative) *m/z*: 151 $[M-H]^-$, 152 $[M]^-$. (Yang *et al.*, 2007)

4-Hydroxybenzaldehyde (**8**) was obtained as colorless crystal, $C_7H_6O_2$; ^{1}H NMR (400 MHz, pyridine-d_5): δ_H 10.00 (1H, s, CHO), 7.94 (2H, d, J=8.0 Hz, H-2, H-6), 7.21 (2H, d, J=8.0 Hz, H-3, H-5); ^{13}C NMR (100 MHz, pyridine-d_5): δ_C 190.8 (d, CHO), 165.0 (s, C-4), 132.8 (d, C-2, C-6), 129.9 (s, C-1), 116.8 (d, C-3, C-5); ESI-MS (positive) *m/z*: 123 $[M+H]^+$, 145 $[M+Na]^+$. (Feng *et al.*, 2008)

Cytotoxic Assay All compounds were tested for their cytotoxicity against A549, HeLa and BGC-823 cancer cell lines with the sulforhodamine B assay (He *et al.*, 2011). Cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% FBS (Tianjin Haoyang) at 37 ℃ and 5% CO_2. After seeded in 96-well microtiter plates for 24 h, cells were treated with serial dilutions of the tested compounds with the maximum concentration of 20 $\mu g \cdot mL^{-1}$. Each compound was initially dissolved in DMSO and further diluted by the medium to produce different concentrations. After incubation for 48 or 72 h, cells were fixed with ice-cold 50% trichloroacetic acid at 4 ℃ for 1 h. With staining for 15 min by 0.4% SRB (Sigma) in 1% glacial acetic acid, excessive dye was removed by washing with 1% glacial acetic acid. Finally, SRB was re-suspended in 10 $mmol \cdot L^{-1}$ Tris buffer and the absorbance was measured at 560 nm with a plate reader (Molecular Devices, SPECTRA MAX 340). The result was expressed as IC_{50} value, which is the concentration that causes 50% growth inhibition. Taxol was used as a reference compound.

DPPH assay The antioxidant activity of all compounds was determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (TCI) method according to the procedure described by us (Adebayo *et al.*, 2010). Each compound solution in dimethyl sulphoxide (DMSO, 5 μL) was added to 195 μL of 0.025 $g \cdot L^{-1}$ DPPH methanol solution. Absorbance at 515 nm was taken after 30 minutes by incubated at room temperature in the microplate reader (Molecular Devices, SPECTRA MAX 340). If the absorbance decreased over 50% by compared with the DMSO control at the concentration of 20 $\mu g \cdot mL^{-1}$, at least four serially diluted concentrations of tested compounds were prepared to evaluate their antioxidant activity using the above procedure. The result was expressed as EC_{50} value, which is the amount to decrease the initial DPPH concentration by 50%. Vitamin C was used as a reference compound.

Acknowledgement: The authors are grateful to the members of the analytical group from the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measuring the [α], IR, UV, NMR and MS spectra.

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